

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 38/18	A1	(11) International Publication Number: WO 96/14081 (43) International Publication Date: 17 May 1996 (17.05.96)
(21) International Application Number: PCT/NL95/00370 (22) International Filing Date: 26 October 1995 (26.10.95) (30) Priority Data: 94203205.3 3 November 1994 (03.11.94) EP (34) Countries for which the regional or international application was filed: AT et al. (71) Applicant (for all designated States except US): BOEHRINGER MANNHEIM GMBH (DE/DE); Sandhofer Strasse 116, D-68298 Mannheim (DE). (72) Inventor; and (75) Inventor/Applicant (for US only): SWAAK, Anthonius, Josef, Gerardus [NL/NL]; Kralingseweg 322, NL-3066 RB Rotterdam (NL). (74) Agent: SMULDERS, Th., A., H., J.; Vereenigde Octrooibureaux, Nieuwe Parklaan 97, NL-2587 BN The Hague (NL).	(81) Designated States: AU, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, JP, KR, LT, LV, MD, MX, NO, NZ, PL, RU, SI, SK, UA, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(54) Title: USE OF ERYTHROPOIETIN IN THE TREATMENT OF RHEUMATOID ARTHRITIS (57) Abstract <p>A novel use of the known protein Erythropoietin (EPO) and/or a derivative and/or a fragment thereof is disclosed. EPO is used as a pharmaceutical for the treatment of chronic inflammations. A particularly beneficial result is seen in patients suffering from rheumatoid arthritis (RA). Significant effects are seen in clinical variables such as morning stiffness, swollen joints, and the like.</p>		

BEST AVAILABLE COPY

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LJ	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

Title: Use of erythropoietin in the treatment of rheumatoid arthritis.

The invention relates to certain novel uses of the known protein erythropoietin (EPO), or substances having such activity as disclosed herein.

5 Erythropoietin is a humoral regulator of erythropoiesis, which stimulates the production of erythrocytes. In normal conditions it is produced in sufficient quantities in the kidneys and the liver.

10 In case of hypoxic shocks (such as massive blood loss) erythropoietin production needs to be increased, which means that it has to be synthesised de novo. In disease-free conditions, erythropoietin levels in circulation are extremely low.

15 Certain diseases or side-effects of treatments of certain diseases lead to a chronic anaemia which overcharges the capacity of erythropoietin production, or otherwise cannot be met by the body's own erythropoietin resources. These diseases include chronic insufficiency of the kidneys, anaemias associated with malignancies, neonate anaemia, chronic anaemia associated with rheumatoid arthritis (ACD), anaemia after bone
20 marrow transplantation, aplastic anaemia, myeloplastic syndrome and various haemoglobin related diseases. Also anaemic side effects have been shown to occur in various chemotherapies and AZT-therapy.

25 In these cases it may be helpful to administer EPO to increase erythrocyte production.

Human EPO is available as a recombinant protein, which ensures that sufficient quantities can be produced in a very pure form.

30 Several studies with recombinant human erythropoietin (r-hu-Epo) have been carried out, mainly in patients who underwent renal dialysis for chronic renal failure, in which diminished production of Epo and severe anaemia requiring regular bloodtransfusions occurs. A correction of anaemia by

r-hu-Epo was shown in these cases with minimal side-effects (16,17,18). In AIDS-patients treated with Zidovudine, causing bone marrow suppression, administration of 100 U r-hu-Epo/kg thrice weekly intravenously, significantly decreased
5 transfusion requirements (19).

The invention provides a novel use of erythropoietin which is not directly related to its erythrocyte stimulating properties.

This use is specifically clear in rheumatoid arthritis, which
10 therefore is more specifically described as explanatory example for the invention.

Rheumatoid arthritis is an inflammatory disease of synovial membranes, usually expressing itself in a symmetrical polyarthritis. During the course of their disease 70% of
15 rheumatoid arthritis (RA) patients develop some kind of anaemia (1), which may be due to iron deficiency (2,3), vitamin B12 deficiency or folic acid deficiency (4,5), haemolysis or adverse reactions to anti-rheumatic drugs (6,7). In addition active RA is frequently (in nearly 50%)
20 accompanied by anaemia of chronic disease (ACD) (8).

Factors involved in the pathogenesis of ACD are ineffective erythropoiesis (9), interleukin-1 (10), tumour necrosis factor α (TNF- α) (11), decreased erythropoietin synthesis (5,12,13) and/or a decreased response to
25 erythropoietin by the bone marrow (14,15).

So far only a few studies with r-hu-Epo have been carried out in RA patients. A haemoglobin (Hb) rise was shown in two anaemic RA patients treated with r-hu-Epo, 125-250 IU/kg thrice weekly, a significant haematocrit rise was recorded
30 (20).

We have treated ten RA patients who suffered from ACD with recombinant human EPO.

In all RA patients a rise in haemoglobin was observed. Despite a wide range of values, the increase in haemoglobin
35 became significant after the second week of treatment with recombinant human EPO.

Besides this expected result of EPO treatment a different unexpected benefit was obtained by the treatment.

The invention thus provides the use of erythropoietin or a substance having erythropoietin-like activity in the preparation of a pharmaceutical for the treatment of chronic inflammations, especially those related to (auto-)immune diseases, in particular RA. In RA we found an overall improvement in the clinical parameters for scoring disease activity. Most impressive are the results on clinical variables such as painscore and morning stiffness as disclosed below. A significant decrease in the number of tender joints was already observed after two weeks of treatment. The changes in other clinical parameters did not reach statistical significance due to the wide range of values and the small number of patients in the study. However, when the parameters were expressed as percentages of their baseline value, significant improvements were observed.

In addition to this effect on clinical variables a further positive effect was seen in the area of an overall sense of well-being of the treated patients.

According to the invention any erythropoietin which has the ameliorating effect on chronic inflammations can be used. Preferably this erythropoietin is not immunogenic so that it can be administered repeatedly. This will usually lead to the use of human erythropoietin of any origin, although recombinant erythropoietin seems the product of choice because of its purity and constant quality. On the other hand it may very well be possible to use non-human truncated forms of mammalian erythropoietin as long as they have the activity and are not immunogenic upon normal administration to patients. Selected mutants (longer acting, more stable), fragments or derivatives of erythropoietin may also be used as long as they fulfil both criteria.

It is worthwhile to note that patients not having a kind of anaemia can thus be treated with EPO. However, caution has to be taken that Hb-levels do not rise to detrimental levels. Ways of lowering the Hb-levels are well-known in the art.

Also, it will be necessary to ensure that no hypertension occurs at a detrimental level. Ways to avoid such a reaction are also well known in the art.

One of the mechanisms through which EPO may ameliorate the disease symptoms in RA (or other chronic inflammations) is that it mobilises iron towards haemoglobin production. Iron (free and/or bound in ferritin) deposits are known to occur in the synovia of RA-affected patients. Synovial fluid iron levels correlate with RA activity and therefore it is thought that iron is involved in the initiation or maintenance of RA synovitis through mediating tissue damage. The role of iron in the pathogenesis of RA may be related to the fact that iron stimulates the production of hydroxyl radicals, which are very potent agents in the destruction of cartilage, membranes and proteins. A thorough discussion of the role and the mechanisms of iron in the inflamed joint can be found in Vreugdenhil et al. (23). In said study it is suggested to administer iron chelators to RA patients. EPO does not chelate iron. However, EPO does mobilise iron to be incorporated into haemoglobin through serum transferrin. Thus EPO may reduce the levels of iron in the synovial fluids.

Another possible mechanism which may be responsible for the unexpected beneficial effect of EPO in (especially) RA, may be found in its influence on the T_H1/T_H2 balance.

One of the key functional parameters determining the outcome of immune responses, for example infectious agents, is the nature of the cytokines produced locally by immune cells. At this moment evidence is obtained that T-cells can be classified into T_H1 and T_H2 cells; both characterized by a different cytokine secretion profile. T_H1 cells secrete IL-2 and TNF- γ upon activation but not IL-4 or IL-5, and T_H2 cells produce IL-4 and IL-5 but not IL-2 or TNF- γ . The differential cytokine profile of these CD4+T cells correlates with different effector functions exerted by these cells: T_H1 cells mediate delayed type hypersensitivity (DTH) responses and T_H2 provide superior help for antibody productions by B cells. There is also some support for the notion that T_H1 and T_H2

cells are progeny of T_H0 cells which can produce IL-2, TNF- γ , IL-4 and IL-5 simultaneously. T_{H1} like cytokine secretion profile. In different animal studies and observations in human diseases, like leprosy, evidence is obtained that the balance
5 between T_{H1} and T_{H2} response determined the outcome of for example an infectious disease and disease manifestations. At this moment a selective activation of T_{H1} -like T cells is proposed as a hallmark of the aethiopathogenesis of rheumatoid arthritis. Evidence for this hypothesis is formed by the fact
10 that on histopathological examination of the synovial tissue, a DTH like of inflammatory reaction is observed which is characteristic for a T_{H1} response.

Some observations in our RA patients treated with r-hu-EPO showed a rise in serum IgE levels; which is consistent
15 with the concept that EPO can give support for a T_{H2} -like response. In other ways influencing the T_{H1} - T_{H2} balance in a more T_{H2} cytokine secretion profile. Indirect evidence for this hypothesis is formed by the fact that 2 out of 3 monoclonals raised against EPO are of the IgE class (IgE synthesis is
20 regulated by IL-4).

When EPO is administered to new-born rats a reduced neutrophil production is observed. This reduced neutrophil production may be partly responsible for the ameliorating effect observed in our patients in that neutrophils play a key
25 role in inflammatory reactions.

It has also been observed that EPO can in some ways counteract the activity of TNF- α . TNF- α is an important pro-inflammatory cytokine.

It may also be the case that EPO diverts the multipotent
30 progenitor blood cells to the production of erythrocytes instead of granulocytes.

EXPERIMENTAL

Patients:

This study focused on the effects of r-hu-Epo on RA
35 disease activity parameters. It is a part of a project studying the pathogenesis of ACD and possible therapeutic

strategies. The effect of r-hu-Epo on the anaemia and iron metabolism is reported in more detail (21).

Ten patients with RA (22) were studied, fulfilling the criteria for ACD as proposed by Carwright (8). ACD was confirmed by measuring stainable iron in a bone marrow preparation. Patients treated previously with iron, vitamin B12, folic acid and cytotoxic drugs were excluded. Other causes of anaemia were also excluded such as the presence of haematuria, positive occult bloodtest in stool, decreased creatinine clearance, haemolysis and low vitamin B12 or folic acid.

The demographic features of the studied patients are summarized in table I. All patients used a variety of non steroidal anti-inflammatory drugs.

15 Treatment:

Recombinant human Erythropoietin (r-hu-Epo, Boehringer, Mannheim, Germany), was administered three times a week at a dose of 240 units/kg subcutaneously at the right upper leg for 6 weeks.

20 Clinical and laboratory monitoring:

Detailed clinical and laboratory evaluation was performed at entry and weekly by the same physician, till the end of the study (6 weeks), then at 9 and 12 weeks after onset of the study. Routine laboratory procedures were used for assessment of haemoglobin (Hb), haematocrit (Ht), mean corpuscular volume (MCV), mean corpus haemoglobin (MCH) and reticulocytes count. Serum iron was measured spectrophotometrically (Instruchemie, Hilversum, the Netherlands). Transferrin and CRP was assessed with a nephelometer (Ablon Medical Systems, Leusden, the Netherlands) and serum ferritin by solid phase enzyme immune assay (Ferrizyme, Abbott Labs, Chigaco, USA). The erythrocyte sedimentation rate (ESR) was measured by the Westergren method. The Ritchie index, grip strength, number of swollen joints, morning stiffness and a subjective pain score (visual analogue scale, 0-10 points) were assessed as well. Liver and kidney function tests were performed to monitor possible side effects.

Data evaluation:

For evaluation all clinical data were stored and analyzed on a Wang personal computer using the Lotus 1-2-3 program.

- 5 Statistical evaluation of the results was by Fishers' exact test for group differences. P values of 0.05 or less were considered significant.

RESULTSEffect of r-hu-Epo on the anemia of chronic disease (ACD).

- 10 In all RA patients a rise in haemoglobin was observed (table II). Despite of the wide range of values, the increase in haemoglobin became significant after the second week of treatment compared to baseline values. When treatment was stopped haemoglobin stayed significant higher compared to the
15 baseline value, but dropped in the 12th week.

Iron deficiency developed as shown by the fact that five patients were characterized by ferritin levels lower than 40 µg/ml.

Effect of r-hu-Epo on disease activity parameters.

- 20 Laboratory parameters: ESR and CRP.

- A decrease in ESR was found in all patients (table III), which started at the third week of treatment and remained so until the end of the study. As illustrated the decrease in eight patients was more than 20% of their baseline value;
25 which was highly significant. The same holds true for the CRP values, but due to the wide range in the absolute values and small number of investigated patients, no significance could be calculated. However, expressing the values as a percentage of the baseline value, also in this way after the third week
30 of treatment, a significant decrease in the CRP levels was observed.

Subjective clinical scores: painscore (PS) and morningstiffness (MS).

- Both parameters (PS and MS) showed during the follow-up a
35 tendency to decrease (table IV). Caused by the variability in absolute values and small number of patients a significance could not be calculated. When the values were expressed in a

percentage of the baseline value, the PS decreased significantly after the third week of treatment and the MS after the sixth week.

Objective disease activity scores: gripstrength (GS),

5 Ritchie Index (RI) and number of swollen joints (SJ).

All parameters as shown in table V showed a continuous tendency towards improvement which lasted during, and also after, the treatment period. In the absolute changes in number of tender joints a significant decrease could be calculated
10 from the third week of treatment. Also a continuous decrease in the number of swollen joints was observed from T3 on and at T9 nine out of ten patients had less swollen joints, which was highly significant.

Caused by the variation of the individual values of the
15 GS, it was impossible to calculate a significance. However, when the values were expressed as a percentage of their baseline values after three weeks of treatment, a significant increase in GS was noted. It should be mentioned that the GS remained stable in three patients during our investigation.

TABLE I

Demographic features of ten patients characterized on having anaemia of chronic disease (ACD) and rheumatoid arthritis (RA)

Female/Male	9/1
Mean age (years)	68 ± 6,5
Treatment:	
Prednisolone	(2 patients) 5 mg 1.5-2.5 g/day
Sulphasalazine	(3 patients) (range) 200 mg/day
Plaquenil	(1 patient) 50 mg/in 2 weeks
Auromyose	(1 patient) 500-750 mg/day
D-Penicillamine	(2 patients) (range)

- 5 All patients were treated for more than 2 months with the mentioned disease modifying anti-rheumatic drugs.

TABLE II

Effect of recombinant human erythropoietin (r-hu-Epo) therapy on haemoglobin and ferritin levels at the defined time periods after onset therapy in ten patients with rheumatoid arthritis (RA)

Variable	Base-line	Values during the 6 weeks therapy and after and 6 weeks of treatment.							
	TO*	T1	T2	T3	T4	T5	T6	T9	T12
Hemo-globin	5.9	6.1	6.5**	6.8	7.0	7.2	7.2	7.2	6.6
mmol/l	0.4	0.5	0.6	0.7	0.9	1.0	1.0	1.1	0.9
± sd									
Ferritin material	216		143**				80	49	61
µg/ml	140-318		44-301				14-157	19-82	52-84
Range									

* Refers to treatment weeknumber.

** Marks the treatment period when the differences between baseline became significant.

TABLE III

Effect of recombinant human erythropoietin (r-hu-Epo) treatment on the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels at the defined time periods after onset therapy in ten patients with rheumatoid arthritis (RA)

Variable	Baseline	Values during 6 and 3 weeks after the end of treatment period.		
		T3*	T6	T9
ESR (mmH)				
mean	82	61**	53**	56**
ranges	42-137	18-112	7-98	7-111
ESR (%)				
mean	100	63	59	64
ranges	-	32-107	16-108	16-144
Number of patients with a change > 20% baseline value	-	8**	7**	8**
CRP (mg/l)				
mean	51	45	43	44
ranges	10-105	4-113	3-122	1-144
CRP (%)				
mean	100	85	85	81
ranges	-	17-155	8-204	5-181
Number of patients with a change > 20% baseline value	-	5**	6**	6**

* Refers to treatment weeknumber.

10 ** Marks the treatment period when the differences compared to baseline values became significant.
P > 0.05, Fishers's exact test.

TABLE IV

Effect of recombinant human erythropoietin (r-hu-Epo) treatment on the overall pain score (PS) and morning stiffness duration (MS) at the defined time periods after onset treatment in ten patients with rheumatoid arthritis (RA).

Variable	Baseline	Values during 6 and 3 weeks after the end of treatment period.		
		T3*	T6	T9
PS				
mean	3.9	3.0	2.7	2.8
ranges	2.7	1-5	1-5	1-5
PS (%)				
mean	100	82	70	73
ranges	-	50-150	33-150	33-100
Number of patients with a change > 20% baseline value	-	7**	8**	6**
MS (min)				
mean	45	37	35	36
ranges	10-120	10-120	10-120	10-120
MS (%)				
mean	100	88	78	85
ranges	-	50-150	50-150	50-150
Number of patients with a change > 20% baseline value	-	3	5**	5**

* Refers to treatment weeknumber.

** Marks the treatment period when the differences compared to baseline values became significant.
P > 0.05, Fishers's exact test.

TABLE V

Effect of recombinant human erythropoietin (r-hu-Epo) treatment on the Ritchie index (RI), number of swollen joints (SJ) and grip strenght (GS) at the defined time periods after onset treatment in ten patients with rheumatoid arthritis (RA).

Variable	Baseline	Values during 6 and 3 weeks after the end of treatment period.		
		T3*	T6	T9
RI mean	13	10.2	7.7**	6**
ranges	3-38	1-22	1-14	2-13
RI (%) mean	100	66	62	56
ranges	-	25-100	33-111	22-95
Number of patients with a change > 20% baseline value	-	8**	7**	9**
SJ mean	8	6	4.5	4.5
ranges	6-5	3-11	2-8	1-9
SJ (%) mean	100	72	61	51
ranges	-	42-100	37-100	20-100
Number of patients with a change > 20% baseline value	-	8*	7*	9*
ESR (mmH) mean	72	87	91	90
ranges	15-190	20-220	20-220	15-220
ESR (%) mean	100	112	118	118
ranges	-	90-133	90-166	90-166
Number of patients with a change > 20% baseline value	-	4**	4**	5**

* Refers to treatment weeknumber.

10 ** Marks the treatment period when the differences compared to baseline values became significant.

P > 0.05, Fishers's exact test.

References

1. Mowat MG: Hematologic abnormalities in rheumatoid arthritis. *Semin Arthr Rheum* 1971; 1:383-390.
- 5 2. Hansen TM, Hansen HE, Birgens HS, Hölund B, Lorenzen I: Serum ferritin and the assessment of iron deficiency in rheumatoid arthritis. *Scand J Rheumatol* 1983; 12:353-359.
3. Vreugdenhil G, Baltus CAM, Van Eijk HG, AJG Swaak: Anemia of chronic disease. Diagnostic significance of erythrocyte and serological parameters in iron deficient rheumatoid arthritis patients. *Br J Rheumatol* 1990; 29:105-110.
- 10 4. Couchman KG, Bieder L, Wigley RD, Glenday AG: Vitamin B12 absorption and gastric antibodies in rheumatoid arthritis. *NZ Med J* 1968; 153-156.
- 15 5. Vreugdenhil G, Wognum AW, Van Eijk HG, AJG Swaak: Anemia in rheumatoid arthritis. The role of iron, vitamin B12 and folic acid deficiency and erythropoietin responsiveness. *Ann Rheum Dis* 1990; 49:93-98.
- 20 6. Van de Putte LBA: Pancytopenia related to azathioprine in rheumatoid arthritis. *Ann Rheum Dis* 1988; 47(6):503-505.
7. Dinant HJ, De Maat CEM: Erythropoiesis and mean cell lifespan in normal subjects and patients with the anemia of rheumatoid arthritis. *Br J Hematol* 1979; 39:437-444.
- 25 8. Cartwright GE and Lee GR: The anemia of chronic disorders. *Br J Hematol* 1971; 21:147-152.
9. Williams RA, Samson D, Tikerpae J, Crowne H, Gumpel JM: In vitro studies of ineffective erythropoiesis in rheumatoid arthritis. *Ann Rheum Dis* 1982; 41:502-507.
- 30 10. Maury CPJ, Andersson LC, Teppo AM, Patanen S, Juronen E: Mechanisms of anemia in rheumatoid arthritis: demonstration of raised interleukin-1 beta concentrations in anemic patients and of interleukin-1 beta mediated suppression of normal erythropoiesis and proliferation of human erythroleukemia cells in vitro. *Ann Rheum Dis* 1988; 47:972-978.
- 35

11. Roodman GD: Mechanisms of erythroid suppression in anemia of chronic disease. *Blood Cells* 1987; 13:171-184.
12. Ward HP, Gordon B, Pickett JC: Serum levels of erythropoietin in rheumatoid arthritis. *J Lab Clin Med* 1969; 74:93-97.
13. Baer AN, Dessypris N, Goldwasser E, Krantz SB: Blunted erythropoietin response to anemia in rheumatoid arthritis. *Br J Hematol* 1987; 66:559-564.
14. Zucker S, Lysik RM, Di Stefano M: Cancer cell inhibition on erythropoiesis. *J Lab Clin Med* 1980; 99:770-782.
15. Harvey AR, Clarke BJ, Chui DHK, Kean F, Buchanan WW: Anemia associated with rheumatoid disease. Inverse correlation between erythropoiesis and both IgM and rheumatoid factor levels. *Arthr Rheum* 1983; 26:28-34.
16. Esbach JW, Egrie JC, Downing MR: Correction of the anemia of endstage renal disease with recombinant human erythropoietin results of a phase I and II clinical trial. *N Eng J Med* 1987; 316:73-78.
- Urabe A, Tokaku F, Mimura N: Therapeutic effect of recombinant human erythropoietin in anemia caused by chronic renal disease. *Exp Hematol* 1987; 15:438-441.
18. Ponticelli C, Casat S: Correction of anemia with recombinant human erythropoietin. *Nephron* 1989; 52:201-208.
19. Fischl M, Galpin JE, Levine JD: Recombinant human erythropoietin for patients with AIDS treated with Zidovudine. *N Eng J Med* 1990; 21:1488-1493.
20. Pincus T, Olsen NJ, Russel IJ: Multicenter study of recombinant human erythropoietin in correction of anemia in rheumatoid arthritis. *Am J Med* 1990; 89:161-168.
21. Vreugdenhil G, Manger B, Nieuwenhuizen C, van Eijk HG, Swaak AJG: Iron stores and serum transferrin receptor levels during recombinant human erythropoietin treatment of anemia in rheumatoid arthritis. *Ann of Hematol* 1992; 65:265-268.

22. Arvett FC, Edworthy S, Bloch DE: The American Rheumatism Association 1987, revised criteria for the classification of rheumatoid arthritis. Arthr Rheum 1988; 31:315-324.
23. Synovial Iron Deposition and Rheumatoid Arthritis.
5 G Vreugdenhil and AJG Swaak in: Handbook on metal-ligand interactions in biological fluids vol. 2 part four, chapter 5 section c. Editor: Guy Berthon: 1993.

CLAIMS

1. Use of erythropoietin or a substance having erythropoietin-like activity in the preparation of a pharmaceutical for the treatment of chronic inflammations.
2. Use according to claim 1, wherein the inflammation is associated with an immune disease.
3. Use according to claim 2 wherein the immune disease is an auto-immune disease.
4. Use according to claim 3, wherein the auto-immune disease is rheumatoid arthritis.
5. Use of erythropoietin or a substance having erythropoietin-like activity in the preparation of a pharmaceutical for the treatment of symptoms associated with rheumatoid arthritis.
6. Use according to claim 5, wherein the symptoms treated comprise at least one of the group of morning stiffness, painful and swollen joints, loss of grip strength and pain.
7. Use of erythropoietin or a substance having erythropoietin-like activity in the preparation of a pharmaceutical for the amelioration of disease activity of rheumatoid arthritis.
8. Use according to anyone of the afore going claims, wherein the erythropoietin is human erythropoietin.
9. Use according to anyone of the foregoing claims wherein the erythropoietin or the substance having such activity is of recombinant origin.

INTERNATIONAL SEARCH REPORT

Inter nal Application No

PCT/NL 95/00370

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB,A,2 171 304 (CHUGAI SEIYAKU K.K.) 28 August 1986 see the whole document ---	7-9
Y	EP,A,0 269 394 (KIRIN-AMGEN, INC.) 1 June 1988 see page 2, line 5 - line 23; claims 1-4 see page 2, line 33 - line 39 see page 2, line 45 - line 47 --- -/--	1-9

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

30 January 1996

Date of mailing of the international search report

15. 03. 96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+ 31-70) 340-3016

Authorized officer

Ryckebosch, A

INTERNATIONAL SEARCH REPORT

Internat Application No
PC1/NL 95/00370

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHEMICAL ABSTRACTS, vol. 101, no. 3, 16 July 1984 Columbus, Ohio, US; abstract no. 21611u, P. BIEMOND ET AL. 'IRON MOBILIZATION FROM FERRITIN BY SUPEROXIDE DERIVED FROM STIMULATED POLYMORPHONUCLEAR LEUKOCYTES. POSSIBLE MECHANISM IN INFLAMMATION DISEASES.' page 446; see abstract & J. CLIN. INVEST., vol. 73, no. 6, 1984 pages 1576-1579,	1-9
Y	ANNALS OF HEMATOLOGY, vol. 65, no. 6, December 1992 NEW YORK, N.Y., US, pages 265-268, G. VREUGDENHIL ET AL. 'IRON STORES AND SERUM TRANSFERRIN RECEPTOR LEVELS DURING RECOMBINANT HUMAN ERYTHROPOIETIN TREATMENT OF ANEMIA IN RHEUMATOID ARTHRITIS.' cited in the application see page 267, left column, line 38 - line 54	1-9
P, X	ARTHRITIS & RHEUMATISM, vol. 38, no. 9(SUPPLEMENT), September 1995 NEW YORK, N.Y., US, page S288 H.R.M. PEETERS ET AL. 'EFFECT OF RECOMBINANT-HUMAN ERYTHROPOIETIN ON ANAEMIA AND DISEASE ACTIVITY IN PATIENTS WITH RHEUMATOID ARTHRITIS AND ANAEMIA OF CHRONIC DISEASE. A LONG-TERM PLACEBO-CONTROLLED DOUBLE-BLIND TRIAL.' see abstract nr. 813	1-9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/NL 95/00370

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB-A-2171304	28-08-86	FR-A- 2576792	08-08-86
		JP-B- 6072103	14-09-94
		JP-A- 62000032	06-01-87
		US-A- 4732889	22-03-88

EP-A-0269394	01-06-88	US-A- 5013718	07-05-91
		AU-B- 602028	27-09-90
		DE-A- 3773852	21-11-91
		IE-B- 60865	24-08-94
		JP-B- 6092316	16-11-94
		JP-A- 63159322	02-07-88
		KR-B- 9509100	14-08-95
		WO-A- 8803808	02-06-88

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.